Blood pressure normalization-independent cardioprotective effects of endogenous, physical activity-induced alpha Calcitonin Gene-Related Peptide (CGRP) in chronically hypertensive mice

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Blood Pressure Normalization-Independent Cardioprotective Effects of Endogenous, Physical Activity-Induced Alpha Calcitonin Gene-Related Peptide (αCGRP) in Chronically Hypertensive Mice

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ABSTRACT

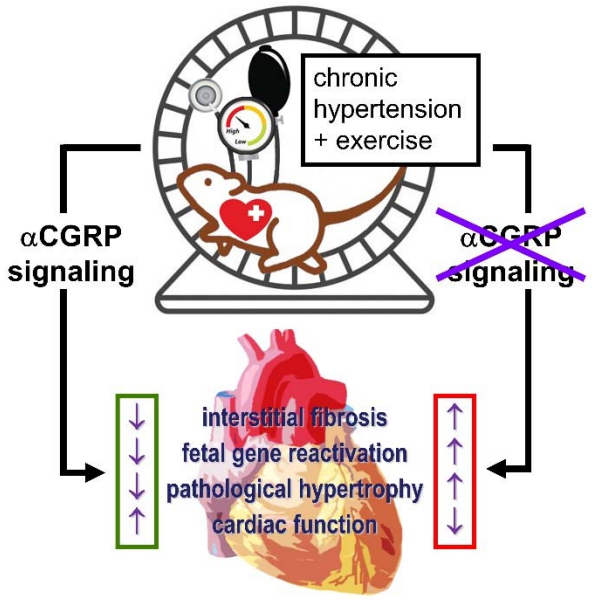
Rationale: α-calcitonin gene related peptide (αCGRP), one of the strongest vasodilators, is cardioprotective in hypertension by reducing the elevated blood pressure (BP).

Objective: However, we hypothesize that endogenous, physical activity-induced αCGRP has BP-independent cardioprotective effects in chronic hypertension.

Methods and Results: Chronically hypertensive (one-kidney-one-clip surgery) WT and αCGRP-/- sedentary or voluntary wheel running mice were treated with vehicle, αCGRP, or the αCGRP receptor antagonist CGRP8-37. Cardiac function and myocardial phenotype were evaluated echocardiographically and by molecular, cellular and histological analysis, respectively. BP was similar among all hypertensive experimental groups. Endogenous αCGRP limited pathological remodeling and heart failure in sedentary, chronically hypertensive WT mice. In these mice, voluntary wheel running significantly improved myocardial phenotype and function, which was abolished by CGRP8-37 treatment. In αCGRP-/- mice, αCGRP treatment, in contrast to voluntary wheel running, improved myocardial phenotype and function. Specific inhibition of proliferation and myofibroblast differentiation of primary, murine cardiac fibroblasts by αCGRP suggests involvement of these cells in αCGRP-dependent blunting of pathological cardiac remodeling.

Conclusions: Endogenous, physical activity-induced αCGRP has BP-independent cardioprotective effects and is crucial for maintaining cardiac function in chronic hypertension. Consequently, inhibiting endogenous αCGRP signaling, as currently approved for migraine prophylaxis, could endanger hypertensive patients.

Keywords:
Exercise, endogenous αCGRP, cardiac remodeling, chronic hypertension.



Nonstandard Abbreviations and Acronyms:

1K1C	one-kidney-one-clip
2',5'-DDA	2',5'-Dideoxyadenosine
AngII	angiotensin-II
AR	aortic rupture
BP	blood pressure
CALCRL	calcitonin receptor-like receptor
Calsq1	calsequestrin-1
CF	cardiac fibroblasts
CI	confidence interval
Col1a1	collagen type-1 α1
Col3a1	collagen type-3 α1
CSA	cross-sectional area
DCP	dilated cardiac phenotype
ddPCR	droplet digital PCR
EF	ejection fraction
EPAC1	exchange protein directly activated by cAMP 1
FS	fractional shortening
H&E	hematoxylin and eosin
HF	heart failure
HW/TL	heart weight to tibia length ratio
KF	kidney failure
LV	left ventricular
Myh	myosin heavy chain
Nppa	atrial natriuretic peptide
Nppb	brain natriuretic peptide
PRD	presumed cardiac rhythm disturbance
RAMP1	receptor activity modifying protein-1
RWT	relative wall thickness
SERCA2	sarcoplasmic reticulum Ca^{2+} ATPase-2
VG	van Gieson's
WGA	wheat germ agglutinin
αCGRP	α-calcitonin gene related peptide
αSMA	α-smooth muscle actin

INTRODUCTION

Chronic hypertension affects more than one billion people worldwide and is the major cause for cardiovascular mortality and morbidity, and the leading global risk factor for heart failure (HF).[1,2] Continuously elevated blood pressure (BP) stimulates cardiomyocytes to increase sarcomere gene transcription resulting in cellular hypertrophy, and cardiac fibroblasts to differentiate into collagen secreting myofibroblasts causing interstitial fibrosis, impairing contractile function and finally resulting in clinical HF.[3,4] Consequently, reduction of pathologically increased BP is currently the most important therapeutic measure, especially if the cause is unknown, as in chronic primary hypertension. However, a significant percentage of hypertensive patients is unresponsive to anti-hypertensive therapy.[5] Evidence indicates that hypertension-induced pathological ventricular remodeling can be manipulated despite persistent pressure stress.[6] Strategies redirecting the pathological cardiomyocyte hypertrophic response towards physiological

hypertrophy and limiting fibrosis might preserve cardiac function and impede progression to HF, in cases where the BP cannot be controlled sufficiently.[3,4,7]

In contrast to hypertension, exercise also induces cardiac hypertrophy but does not impair heart function or lead to HF. Previous hypotheses suggest that the chronicity (hypertension as continuous vs exercise as intermittent) of a stimulus determines the kind of cardiac hypertrophy. However, a recent study has shown that the nature of the stimulus is important for the hypertrophy phenotype.[8] One mechanism behind this observation is systemic α-calcitonin gene related peptide (αCGRP) signaling, since exercise induces a pathological cardiac phenotype, similar to that seen in hypertension, in αCGRP-deficient mice.[9] In line with this, several independent studies showed that plasma αCGRP concentrations increase in tight correlation to lactate during exercise.[10-14] In working muscles, lactate and low pH activate the metaboreceptor transient receptor potential vanilloid in group III afferent (A-δ) nerves which mediate the well-established exercise pressor reflex[15] and cause neuronal exocytosis of αCGRP.[10,16-18] αCGRP interacts with the receptor formed of calcitonin receptor-like receptor (CALCRL), receptor activity modifying protein-1 (RAMP1) and receptor component protein. It activates cAMP-dependent signaling pathways that protect cardiomyocytes from stress-induced apoptosis[19], and induce physiological cardiomyocyte growth and positive inotropy.[9,20,21] Moreover, exercise antagonizes hypertension-induced pathological cardiomyocyte growth and reduces myocardial fibrosis[22], and is therefore recommended in chronic hypertensive patients with symptomatic HF.[23] Intravenous administration of αCGRP delays the onset of myocardial ischemia upon exercise in patients with stable angina pectoris[24] and improves cardiac function in patients with congestive HF.[25,26] A recent study demonstrated that systemic administration of a long-lasting αCGRP-analogue reduces the increased BP and consequently improves cardiac function in murine models of hypertension and HF.[27]

Here, we tested our hypothesis that exercise-induced, endogenous αCGRP suppresses hypertension-induced pathological cardiac remodeling in the one-kidney-one-clip (1K1C) murine model of chronic hypertension.[28,29] Voluntary cage wheel running was chosen as exercise intervention since it avoids physical and psychological stress caused by forced exercise.[30] Myocardial effects of exercise-induced αCGRP agonism in chronic hypertension were investigated by assessing the cardiac function echocardiographically, and pathological ventricular remodeling by analyzing the cardiac gene expression signature, cardiomyocyte hypertrophy and interstitial fibrosis. We found that endogenous αCGRP is crucial for the beneficial cardiac effects of exercise in chronic hypertension without reducing the associated elevated BP. Of note, blocking the basal αCGRP signaling with a CGRP receptor antagonist worsened the myocardial phenotype and function in sedentary WT mice. Thus, as also suggested by others[27], we consider that activating the αCGRP pathway could have therapeutic potential in chronic hypertension. Further, our study reveals severely impaired left ventricular (LV) function as a potentially life-threatening adverse effect of long-term endogenous αCGRP inhibition in the setting of chronic hypertension. The FDA has recently approved αCGRP antagonists for migraine prophylaxis.[31,32] Therefore, our data are important as we present the potential cardiovascular risk of long-term αCGRP antagonism

METHODS

The authors declare that all supporting data are available within the article and its online supplementary files.

Animals.
Male C57BL/6J WT and αCGRP deficient (αCGRP-/-, characterized previously[33]) mice with the same genetic background, and normal calcitonin gene expression and baseline BP[33], aged 14 to 16 weeks were used. All experiments were conducted in accordance with institutional and governmental guidelines (Supplemental Methods) and approved by the Cantonal Veterinary Department, Zürich, Switzerland.

1K1C hypertension model.
Hypertension was induced by clamping the left renal artery with an U-shaped stainless steel clip and removing the right kidney as described[28,29] (Supplemental Methods).

Exercise interventions and treatments.
To ensure chronic hypertension[29], mice were assigned to the experimental groups 8 weeks after 1K1C micro-surgery by single housing them with or without access to computerized cage wheel running for additional 4 weeks (Supplemental Methods). Simultaneously, mice were treated with vehicle (10% dimethyl-sulfoxide in PBS), αCGRP (4 nM/h) or CGRP8-37 (80 nM/h) using micro-osmotic pumps that were replaced once after 14 days to cover the entire 4 weeks of the treatment period. Initially, the stability of αCGRP and CGRP8-37 in micro-osmotic pumps at 37°C for 14 days (Supplemental Methods, Online Figure I) and the increase in plasma αCGRP concentrations upon αCGRP peptide treatment was verified (Online Table I).

Echocardiography and LV phenotype classification.
Assessment of cardiac dimensions and systolic LV function in M-mode images of the LV in a parasternal short axis view were carried out using a Vevo 2100, Visualsonics system and the data were analyzed using Vevo 2100 software (Supplemental Methods). To classify the myocardial phenotypes observed, the upper 95% confidence interval (CI) of the mean of relative wall thickness (RWT) and heart weight to tibia length ratio (HW/TL) of sham-operated WT and αCGRP-/- mice were determined (Online Table II). The RWT and HW/TL of each individual 1K1C-operated mouse was then determined, resulting in assignment to one of the five myocardial LV structural patterns (Supplemental Methods, Online Figure II). This classification scheme was modified (to become mouse specific) from that described in previous studies[4] and is in accordance with the American Society of Echocardiography's recommendations on defining LV myocardial phenotypes. As none of the mice with chronic hypertension induced by 1K1C in our study showed 'normal' and 'concentric remodeling', and only two mice showed 'eccentric hypertrophy', only the data from animals exhibiting 'concentric hypertrophy' or 'dilated cardiac phenotype' (i.e. excluding the two mice with 'eccentric hypertrophy') are shown in the results.

BP measurements.
BP measurements were performed by femoral artery catheterization as described[9] (Supplemental Methods).

Droplet digital PCR (ddPCR) analysis.
Total RNA isolated from heart tissues and cardiac fibroblasts (CF) were reverse transcribed into cDNA and ddPCR was performed as described[9] (Supplemental Methods). Sequence specific Taqman PCR gene expression probes (target species: mouse) used and their Assay ID are listed in supplemental methods.

Histology.
Heart tissue sections were stained with hematoxylin and eosin (H&E) for histopathology and morphometry of arterioles and small arteries, van Gieson's for visualization of collagen fibres, CF™488-conjugated

wheat germ agglutinin for delineating plasma membranes and quantifying cardiomyocyte cross-sectional area, and DyLight™649-conjugated GSL isolectin-B4 for measurement of capillary to myocyte ratio. Images were acquired using an Axio Imager.Z2. microscope equipped with a digital camera and the AxioVision Rel. 4.8 software, and analyzed using MCID Analysis software (Supplemental Methods, Online Figures III-V).

Mice that either were euthanized due to signs of kidney failure (KF) or died suddenly without signs of impaired well-being following 1K1C were subjected to routine autopsy and histopathology (Supplemental Methods).

Collagen quantification.
Total collagen and protein contents of the heart sections, and cultured CF were spectrophotometrically quantified using Sirius red/Fast green Collagen Staining kit as described[9] (Supplemental Methods).

Blood plasma analysis.
Plasma αCGRP, angiotensin-II (AngII), renin-1, triglyceride and free fatty acid concentrations were quantified using mouse-specific EIA or ELISA kits (Supplemental Methods).

Cell culture experiments.
Adult murine CF were isolated and cultured in DMEM/F-12 supplemented with 10% FBS and 1% penicillin-streptomycin as described[34] with some modifications (Supplemental Methods). Cell proliferation was quantified by measuring BrdU incorporation during DNA synthesis, and cAMP and exchange protein directly activated by cAMP 1 (EPAC1) concentrations in cell lysates were determined using a cAMP and EPAC1 ELISA Kits and normalized for cellular total protein according to the manufacturer's instructions (Supplemental Methods). Immunofluorescence staining of α-smooth muscle actin (αSMA) and collagen was performed with appropriate negative controls (secondary antibody only) as described[39] with modifications (Supplemental Methods). Images were captured using an Axio Imager.Z2. microscope equipped with a digital camera and associated AxioVision Rel.4.8 software.

Statistics.
Data were analyzed using GraphPad Prism software version 8.0, and tested for normality by Shapiro-Wilk test and homogeneity of variances. When parametric assumptions were met, an unpaired Student's *t*-test was used for comparing two independent groups and one-way ANOVA followed by Bonferroni post hoc test was used for comparing multiple groups. Student's *t*-test was used only when the specific research question involved comparing two independent groups (with parametric assumptions met). When parametric assumptions were not met, Kruskal-Wallis followed by Dunn's multiple comparison test was used. While we corrected for multiple testing in post-hoc comparisons, we did not correct across tests. The survival data were plotted using the Kaplan-Meier method and the comparison was performed with the Mantel-Cox test. Differences were considered statistically significant at $P<0.05$.

RESULTS

Since 1K1C surgery results in stable hypertension by the 4th week[28] after surgery and chronic hypertension is established at the latest by the 8th week[29], all WT and αCGRP-/- mice that underwent 1K1C surgery were single housed for 8 weeks under standard conditions. Mice were then housed with or without access to voluntary cage wheel running and treated simultaneously with vehicle, αCGRP, or αCGRP receptor antagonist CGRP8-37 for additional 4 weeks.

αCGRP deficiency impairs survival in chronic hypertension.

Following 1K1C surgery, αCGRP-/- mice exhibited significantly reduced survival compared with WT mice. Kidney failure (KF) and aortic rupture (AR) occurred in 9% and 4% of WT mice after 1K1C surgery, respectively and 87% WT mice survived up to the study end point. In contrast, KF, AR and dilated cardiac failure were observed in 31%, 17% and 6% of αCGRP-/- mice, respectively and 38% of αCGRP-/- mice survived up to the study end point. In 8% of 1K1C-operated αCGRP-/- mice, the cause of death could not be determined by the post-mortem analysis and therefore were presumed to be resulting from cardiac rhythm disturbances (Figure 1).

αCGRP deficiency or αCGRP antagonism increases the occurrence of dilated cardiac phenotype in chronic hypertension.

After 4 weeks of treatment, the LV myocardial structural pattern of each individual 1K1C-operated mouse was defined according to the classification scheme outlined in supplemental methods and Online Figure II. All vehicle- and αCGRP-treated WT mice in both sedentary and voluntarily running groups had increased RWT and HW/TL (Figure 2A, B, blue dots), and were classified as exhibiting a concentric hypertrophy phenotype (Figure 2C). In contrast, only 66.66% of antagonist-treated WT mice in sedentary and 71.43% of antagonist-treated WT mice in voluntarily running groups had increased RWT and HW/TL (Figure 2A, B, blue dots), and were classified as exhibiting a concentric hypertrophy phenotype (Figure 2C). The remaining 33.33% of antagonist-treated WT mice in sedentary and 28.57% of antagonist-treated WT mice in voluntarily running groups had decreased RWT and increased HW/TL (Figure 2A, B, red dots), and were classified as exhibiting a dilated cardiac phenotype (Figure 2C). In line with this, 66.67% of sedentary, vehicle-treated αCGRP-/- mice, 66.67% of voluntarily running, vehicle-treated αCGRP-/- mice, 88.89% of sedentary, αCGRP-treated αCGRP-/- mice, and 77.78% of voluntarily running, αCGRP-treated αCGRP-/- mice showed increased RWT and HW/TL (Figure 2A, B, blue dots), and were classified as exhibiting a concentric hypertrophy phenotype (Figure 2C). The remaining 33.33% of sedentary, vehicle-treated αCGRP-/- mice, 33.33% of voluntarily running, vehicle-treated αCGRP-/- mice, 11.11% of sedentary, αCGRP-treated αCGRP-/- mice, and 22.22% of voluntarily running, αCGRP-treated αCGRP-/- mice showed decreased RWT and increased HW/TL (Figure 2A, B, red dots), and were therefore classified as exhibiting a dilated cardiac phenotype (Figure 2C).

At the end of the treatment period, all 1K1C-operated mice were, in general, similarly hypertensive. Only in some mice with dilated cardiac phenotype, systemic BP was not higher than that measured in sham animals (Figure 2D, Online Table III), possibly indicating cardiac decompensation and impending failure.

αCGRP deficiency impairs voluntary wheel running activity and exacerbates pathological cardiomyocyte hypertrophy in chronic hypertension.

Compared with vehicle-treated hypertensive WT mice, vehicle-treated hypertensive αCGRP-/- mice exhibited significantly reduced cage wheel running. Treatment with αCGRP significantly increased cage wheel running in hypertensive WT and αCGRP-/- mice (Figure 3A- C). Sham-operated WT and αCGRP-/- mice showed similar levels of voluntary wheel running activity (Online Figure VI), thereby proving that global αCGRP deficiency adversely affected wheel running activity only in the setting of chronic hypertension. Directly after 7 minutes of continuous voluntary running, plasma αCGRP concentrations were significantly increased in naive, normotensive (+40%) and vehicle-treated hypertensive WT (+25%) mice compared with their respective sedentary controls (Figure 3D, E).

Voluntarily running, vehicle-treated and sedentary, αCGRP-treated hypertensive WT mice showed significantly decreased cardiomyocyte hypertrophy compared with sedentary, vehicle-treated hypertensive WT mice. In contrast, antagonist (CGRP8-37) treatment significantly increased cardiomyocyte hypertrophy

in voluntarily running hypertensive WT mice. Similarly, cardiomyocyte hypertrophy was significantly increased in sedentary, vehicle-treated hypertensive αCGRP-/- compared with sedentary, vehicle-treated hypertensive WT mice. This suggests that impairment of even basal systemic αCGRP signaling increases cardiomyocyte hypertrophy. Accordingly, treatment with αCGRP significantly suppressed cardiomyocyte hypertrophy in sedentary, and voluntarily running hypertensive αCGRP-/- mice (Figure 4). Morphometry of cardiac vessels showed that αCGRP deficiency, agonism or antagonism did not significantly alter capillary to myocyte ratio (Online Figure VIIa). Arteriolar or arterial structure in chronic hypertensive mice were changed slightly and unsystematically in a few groups only (Online Figure VIIb-j). Thus, angiogenesis or other changes of the cardiac vasculature may not have contributed to the observed protective effect of αCGRP.

Physical activity-induced endogenous αCGRP signaling improves ejection fraction (EF) and fractional shortening (FS) in chronic hypertension.

Echocardiography revealed significantly improved EF and FS in voluntarily running, vehicle-treated and sedentary, αCGRP-treated hypertensive WT mice compared with sedentary, vehicle-treated hypertensive WT mice. Antagonist (CGRP8-37)-treated sedentary mice showed lower EF and FS compared with their vehicle-treated hypertensive WT counterparts (Figure 5). CGRP8-37 treatment significantly and completely abolished the positive effects of voluntary running on cardiac function in voluntarily running hypertensive WT mice. Sedentary, vehicle-treated hypertensive αCGRP-/- mice showed significantly decreased EF and FS compared with sedentary, vehicle-treated hypertensive WT mice. Compared with vehicle treatment, αCGRP treatment significantly increased EF and FS in both sedentary and voluntarily running hypertensive αCGRP-/- mice. Note that combining αCGRP treatment with exercise, compared with sole αCGRP treatment, did not further improve EF and FS in hypertensive αCGRP-/- mice. Voluntarily running, vehicle-treated hypertensive αCGRP-/- mice had, only by trend, a slightly increased EF and FS compared with sedentary, vehicle-treated hypertensive αCGRP-/- mice. Cardiac function was more impaired in the dilated hearts compared with the concentric phenotype in the respective groups (Figure 5). The complete echocardiography data are given in Online Table VI.

Physical activity-induced endogenous αCGRP signaling suppresses hypertension-induced myocardial fetal gene expression signature.

The adult heart responds to hypertension by fetal gene reinduction including isoform switching of sarcomeric myosin heavy chain (Myh, decreased Myh6 and increased Myh7 expression), upregulation of atrial (Nppa) and brain (Nppb) natriuretic peptides, and downregulation of sarcoplasmic reticulum Ca^{2+} ATPase-2 (SERCA2).[36] Compared with sedentary, vehicle-treated hypertensive WT mice, voluntarily running, vehicle-treated and sedentary, αCGRP-treated hypertensive WT mice exhibited significantly higher Myh6 and SERCA2, and lower Myh7, Nppa, and Nppb expression (Figure 6). Antagonist (CGRP8-37) treatment significantly and completely suppressed the positive effects of exercise on myocardial fetal gene expression signature in voluntarily running hypertensive WT mice. Sedentary, vehicle-treated hypertensive αCGRP-/- mice showed significantly decreased Myh6 and SERCA2, and upregulated Myh7, Nppa, and Nppb expression compared with sedentary, vehicle-treated hypertensive WT mice. This suggests that impairment of even basal systemic αCGRP signaling worsens the cardiac fetal gene expression signature in chronic hypertension. Treatment with αCGRP significantly upregulated Myh6 and SERCA2, and downregulated Myh7, Nppa, and Nppb expression in sedentary, and voluntarily running hypertensive αCGRP-/- mice. Voluntarily running, vehicle-treated hypertensive αCGRP-/- mice showed no significant expression difference in Myh6, SERCA2, Myh7, and Nppb compared with sedentary, vehicle-treated hypertensive αCGRP-/- mice. In CGRP8-37-treated hypertensive WT, and vehicle- or αCGRP-treated hypertensive αCGRP-/- mice in both sedentary and voluntarily running groups, fetal Myh isoform switching was prominent and changes in the expression of Nppa, Nppb and SERCA2 were more adversely affected in the dilated hearts compared with the concentric phenotype (Figure 6).

Taken together, these data indicate that in chronic hypertension, global αCGRP deficiency or αCGRP receptor antagonism aggravates the fetal gene expression signature of the pathological cardiac hypertrophy in the absence of exercise, and furthermore, suppresses the positive effects of exercise. Conversely, the expression signature of pathological cardiac hypertrophy can be suppressed by either exercise or αCGRP treatment.

Physical activity-induced endogenous αCGRP signaling suppresses hypertension-induced myocardial fibrosis and myofibroblast differentiation.

Interstitial fibrosis that occurs in hypertension is a key feature distinguishing physiological from pathological myocardial remodeling.[37] Interstitial fibrosis and cardiac collagen level were significantly decreased in voluntarily running, vehicle-treated, and sedentary, αCGRP-treated hypertensive WT mice compared with sedentary, vehicle-treated hypertensive WT mice. In contrast, antagonist (CGRP8-37) treatment significantly and completely abolished the beneficial effects of exercise on interstitial fibrosis and collagen level in voluntarily running hypertensive WT mice. Sedentary, vehicle-treated hypertensive αCGRP-/- mice showed notably increased interstitial fibrosis and collagen level compared with sedentary, vehicle-treated hypertensive WT mice. This suggests that impairment of even basal systemic αCGRP signaling increases hypertension-induced cardiac fibrosis. Accordingly, αCGRP treatment notably suppressed interstitial collagen deposition in sedentary, and voluntarily running hypertensive αCGRP-/- mice. Interstitial fibrosis and collagen level were higher in dilated hearts compared with the concentric phenotype in the respective groups (Figure 7).

Cardiac fibroblasts (CF) are the major cell type secreting collagen during hypertension.[3] We tested *in vitro* if adult murine CF respond specifically to αCGRP treatment. Treatment with αCGRP significantly increased cAMP production in CF (Figure 8A, Online Figure VIII). To elucidate αCGRP's direct effects on collagen synthesis by CF, we stimulated CF with the potent pro-fibrotic hypertensive peptide AngII in the absence or presence of αCGRP. Expression of collagen (Figure 8B- D), and αSMA (encoded by Acta2, Online Figure IXa, b), the molecular markers of pro-fibrotic myofibroblast differentiation, were highly increased upon AngII treatment, in accordance with previous findings.[3] In addition, proliferation of CF, another profibrotic process[38], was also significantly stimulated by AngII (Figure 8E). These pro-fibrotic effects of AngII, i.e. the induction of collagen (Figure 8B- D) and αSMA (Online Figure IXa, b) expression, and cell proliferation (Figure 8E) were significantly suppressed in CF when these cells were treated with a combination of AngII and αCGRP, which in turn was reversed by CGRP8-37 treatment.

AngII induces profibrotic gene expression and myofibroblast transformation by transcriptional downregulation of EPAC1 in CF. Accordingly, it is shown that upregulating EPAC1 expression by enhancing cyclic AMP release can inhibit AngII-induced profibrotic transformation of CF.[39,40] AngII downregulated EPAC1 expression in CF, thereby confirming previous findings.[39,40] Combining AngII with αCGRP, the latter being a cAMP inducer[41] (Figure 8A), significantly upregulated EPAC1, and this effect was blocked by the cAMP synthesis inhibitor 2',5'-Dideoxyadenosine (2'5-DDA[40], Figure 8F, Online Figure IXc). Moreover, treatment with 2'5-DDA, and the EPAC1 specific inhibitor CE3F4[42] abolished αCGRP's beneficial effects on AngII-induced collagen (Figure 8B-D) and αSMA (Online Figure IX) synthesis, and cell proliferation (Figure 8E).

This combination of *in vivo* and *in vitro* data clearly suggests that hypertension-induced cardiac fibrosis is aggravated in the absence of αCGRP or by the inhibition of systemic αCGRP signaling. At the cellular level, the cAMP effector EPAC1 regulates the direct antifibrotic effects of αCGRP in CF. *In vivo*, αCGRP treatment inhibits hypertension-induced cardiac fibrosis, a key feature of pathological heart remodeling. Exercise suppressed interstitial cardiac fibrosis in WT mice but not in αCGRP-/- mice. This

effect in WT mice could be inhibited by CGRP8-37, thereby suggesting that inhibition of cardiac fibrosis by exercise is αCGRP-dependent.

αCGRP did not significantly alter plasma AngII concentrations (Online Figure Xa), thereby excluding a direct modulatory effect of αCGRP on circulating AngII concentration in hypertensive mice. However, αCGRP deficiency slightly increased plasma renin-1 concentrations (Online Figure Xb). This finding of unaltered plasma AngII despite increased plasma renin-1 concentrations, and the complex interaction of αCGRP with renin-angiotensin-aldosterone system during hypertension warrants further investigation.

Although exercise-induced or injected αCGRP promotes lipolysis in normotensive rats.[43], αCGRP did not alter plasma free fatty acid and triglyceride concentrations in our setting (Online Figure X), thereby making altered cardiac fatty acid supply as indirect cause for αCGRP's cardioprotective effects unlikely.

DISCUSSION

Modern antihypertensive therapy includes suggestions for lifestyle changes, of which increased physical activity is a centrally important cornerstone.[44] Exercise improves cardiac function even when hypertension is not significantly reversed.[45] A recent multicenter study showed that little regular physical activity such as walking for about 30 min per day, is the most important independent factor maintaining cardiovascular health.[46] Based on the findings from HF-ACTION trial that investigated the effects of exercise in about 2300 human subjects with HF[23], exercise has been recommended as part of cardiac rehabilitation even when EF is reduced.[37] It is not completely understood yet why exercise is beneficial but our study might provide some explanation to help answer this question.

The αCGRP receptors are constitutively present in the myocardium[9,47], and αCGRP agonism or antagonism did not alter their cardiac expression in the present study (Online Figure VIII). Previous studies showed that exercise prevents fetal gene reactivation and fibrosis in the heart, thereby keeping the heart in an adaptive phase during persistent pressure load.[22] Accordingly, we found suppressed fetal gene reactivation and myocardial fibrosis together with a better heart function (Figure 5) in voluntarily running, vehicle-treated hypertensive WT mice compared with their sedentary counterparts. These cardioprotective effects of exercise were negated by treatment with the αCGRP receptor antagonist CGRP8-37. CGRP8-37 treatment worsened pathological myocardial remodeling also in sedentary hypertensive WT mice because these mice without access to a running wheel still had basal physical activity. Basal plasma αCGRP concentrations may result from an overspill of αCGRP from sensory neurons.[41] After induction of hypertension, αCGRP-/- mice had considerably more cardiovascular events and were affected with a dilated cardiac phenotype compared with hypertensive WT mice (Figure 1B). Accordingly, hypertensive αCGRP-/- mice (sedentary, vehicle-treated) had signs of severe pathological cardiac remodeling and worse cardiac function compared with their WT counterparts. Moreover, the dilated cardiac phenotype with severely impaired cardiac function and enhanced fibrosis, seen in all hypertensive groups of αCGRP-/- mice, occurred in hypertensive WT mice only when WT were treated with CGRP8-37. Thus, impaired αCGRP signaling may worsen pathological cardiac remodeling and induce the dilated cardiac phenotype with reduced LV function and failure. Other αCGRP-/- mice with combined deletion of αCGRP and calcitonin, and altered baseline cardiovascular variables apparently due to calcitonin deficiency[41] exhibit reduced survival and cardiac function upon pressure stress.[48,49] Importantly, our study shows that hypertensive αCGRP-/- mice with unaltered calcitonin expression[33] have reduced survival, and failed to improve cardiac fetal gene expression profile, fibrosis and cardiac function by voluntary running alone. In contrast, αCGRP treatment resulted in considerable reduction of the cardiac fetal gene expression reactivation and myocardial fibrosis, and improvement of cardiac function in hypertensive αCGRP-/- mice, without further

improvement from simultaneous exercise. Improvement of the myocardial phenotype and cardiac function induced by voluntary running in WT mice is due to transient exercise-induced increase in plasma αCGRP concentration (Figure 3D, E), since this was abolished after systemic CGRP8-37 treatment. Importantly, the αCGRP treatment used in this study did not alter the degree of elevated BP in any of the experimental groups (Figure 2D), thereby suggesting that the observed effects are BP-independent. The latter notion is important because, to the best of our knowledge, the cardioprotective effects shown in previous studies employing αCGRP treatment during systemic hypertension were accompanied by reduction of elevated BP.[27,50,51] Further, our data of unaltered structure of cardiac vasculature in response to αCGRP treatment and αCGRP deficiency or antagonism in hypertension suggest that improvements of the cardiac microcirculation may not have contributed to the observed protective effects of αCGRP. The in vitro observation that αCGRP activates the cAMP effector EPAC1 to suppress myofibroblast differentiation and CF proliferation suggests direct antifibrotic effect of αCGRP during hypertension-induced cardiac remodelling. This is in accordance with previous reports showing αCGRP's protective effects on various cells including cardiomyocytes in vitro.[41] Our finding that αCGRP has, in addition to its vasodilating properties, direct hormonal effects on the heart at relatively low plasma concentrations is supported by the notion that is the stimulus and not its chronicity what determines the myocardial phenotype.[8,9] Moreover, pregnancy, a more chronic cardiac stimulus than exercise, is associated with physiological cardiac hypertrophy[52] and, markedly increased plasma αCGRP concentrations.[53] The pregnancy-induced physiological cardiac hypertrophy disappears after delivery simultaneously with normalization of the elevated plasma CGRP concentrations.[52,53]

It is debated whether endogenous plasma αCGRP concentrations are high enough or can reach sufficient levels to mediate cardioprotection under certain circumstances. Normal plasma αCGRP concentrations range from 10-250 pg/mL in mice[54-56] and around 200 pg/mL in humans.[31] After a maximal exercise test in man, plasma αCGRP concentrations were increased by nearly 30%.[10] A recent study reported approximately 70% increase in plasma αCGRP concentrations after forced exercise (26 m min^{-1} for 60 min) in rats[43] that stimulated adipose tissue lipolysis during exercise, an effect that was inhibited by CGRP8-37 pretreatment. This supports our findings that exercise-induced αCGRP exerts hormonal effects. However, even exercise-independent plasma αCGRP appears to exert cardioprotection, especially during chronic hypertension because cardiac phenotype and function were worse in sedentary, vehicle-treated hypertensive αCGRP-/- mice and sedentary, CGRP8-37-treated WT mice compared with sedentary, vehicle-treated WT mice (Figures 2, 4-7). This strongly suggests that even basal plasma αCGRP concentrations (Online Table I), most likely due to overspill from normal peripheral neuronal activity[41], are important for maintaining a healthy heart function in situations of cardiac stresses such as chronic hypertension. Moreover, the shortly peaking αCGRP concentrations might be the key explaining the beneficial cardiovascular effects of exercise in healthy people, hypertensive patients and those with impaired heart function.[37] This notion is well-supported by our finding that plasma αCGRP is significantly increased already after 7 minutes of voluntary wheel running (Figure 3D, E) and that cardioprotective effects of exercise are completely abolished by CGRP8-37 treatment in mice.

Here, we used a mouse model of chronic hypertension (not induced by genetic modification) with a total of 84 days duration, which is longer than many described in the literature (median duration: 28 d, mean duration: 49±45 d, Online Table X). Implanting AngII-filled micro-osmotic pumps, the most common model used for creating systemic hypertension, is limited by the fact that in mice, only small pumps (maximum 4 weeks duration of drug delivery) can be used. In order to address the effects of lifestyle changes, e.g. increased physical activity, longer duration chronic hypertension models appear to be clinically more relevant. The same holds also for the fact that in clinical reality, therapy typically starts not simultaneously with, or just shortly after, onset of hypertension, as in many previous basic studies (Online Table X), but rather when the patient has lived with the problem for a considerable time. Accordingly, we started the treatment and exposed the mice to voluntary wheel running after 8 weeks of hypertension, and treated them for another 4 weeks. Our results support previous observations that even after a longer time of

sedentary lifestyle, increasing the level of physical activity is still effective in hypertensive patients.[45,57] As we show here, these beneficial effects of exercise are independent of normalizing the elevated BP. This clearly reinforces the notion that patients that do not respond to pharmaceutical antihypertensive therapy or have signs of HF can profit from increased physical activity.[37,45] The data presented here and in our previous work[9] show that one mediator of the beneficial cardiac effects of exercise is αCGRP. Therefore, αCGRP analogues could be useful, especially in hypertensive patients with additional co-morbidities that are common in elderly patients, thus, preventing them from taking part in or strictly adhering to suggested exercise regimens.

On the other hand, αCGRP plays a crucial role in the pathophysiology of migraine, a debilitating neurovascular disorder affecting about 16% of the population worldwide. Therefore, antibodies with long biological half-life (> 45 days) against αCGRP or its receptor have recently been approved for clinical use.[32,58] Longtime experience with αCGRP-antagonists for migraine prophylaxis are missing. However, our data suggest that chronic inhibition of endogenous αCGRP signaling might induce adverse cardiovascular effects, especially in hypertensive individuals (that were not included in the original clinical trials for αCGRP antagonists).

Taken together, we demonstrate here for the first time that (1) in mice, the basal, exercise-independent plasma αCGRP is an important survival factor in hypertension, (2) additional cardioprotective effects of voluntary exercise, when started at the stage of stable chronic hypertension are αCGRP-dependent, and (3) αCGRP is cardioprotective also independent of its BP normalization effects. Thus, endogenously-activated αCGRP maintains cardiac health and is crucial for mediating cardioprotective effects of exercise during persistent pressure load. In future, αCGRP agonists might, therefore, replace exercise in clinical conditions where patients are mobility impaired or it is contraindicated. Moreover, we have found that long-term αCGRP antagonism leads to life threatening cardiac impairment during chronic systemic hypertension that cannot be reversed by additional exercise. This warrants attention regarding the cardiac function in individuals who already have hypertension, or may develop hypertension later in life, while being treated with αCGRP antagonists for migraine prophylaxis.

DISCLOSURES

The authors declare no competing interests.

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REFERENCES

1. Mills KT, Bundy JD, Kelly TN, Reed JE, Kearney PM, Reynolds K, Chen J, He J. Global Disparities of Hypertension Prevalence and Control: A Systematic Analysis of Population-Based Studies From 90 Countries. *Circulation*. 2016;134:441-450.
2. Worldwide trends in blood pressure from 1975 to 2015: a pooled analysis of 1479 population-based measurement studies with 19.1 million participants. *Lancet*. 2017;389:37-55.
3. Burchfield JS, Xie M, Hill JA. Pathological ventricular remodeling: mechanisms: part 1 of 2. *Circulation*. 2013;128:388-400.
4. Drazner MH. The progression of hypertensive heart disease. *Circulation*. 2011;123:327-334.
5. Carey RM, Calhoun DA, Bakris GL et al. Resistant Hypertension: Detection, Evaluation, and Management: A Scientific Statement From the American Heart Association. *Hypertension*. 2018;72:e53-e90.
6. Hill JA, Karimi M, Kutschke W, Davisson RL, Zimmerman K, Wang Z, Kerber RE, Weiss RM. Cardiac hypertrophy is not a required compensatory response to short-term pressure overload. *Circulation*. 2000;101:2863-2869.
7. Sano M, Schneider MD. Still stressed out but doing fine: normalization of wall stress is superfluous to maintaining cardiac function in chronic pressure overload. *Circulation*. 2002;105:8-10.
8. Perrino C, Naga Prasad SV, Mao L, Noma T, Yan Z, Kim HS, Smithies O, Rockman HA. Intermittent pressure overload triggers hypertrophy-independent cardiac dysfunction and vascular rarefaction. *J Clin Invest*. 2006;116:1547-1560.
9. Schuler B, Rieger G, Gubser M et al. Endogenous alpha-calcitonin-gene-related peptide promotes exercise-induced, physiological heart hypertrophy in mice. *Acta Physiol (Oxf)*. 2014;211:107-121.
10. Hasbak P, Lundby C, Olsen NV, Schifter S, Kanstrup IL. Calcitonin gene-related peptide and adrenomedullin release in humans: effects of exercise and hypoxia. *Regul Pept*. 2002;108:89-95.
11. Schifter S, Breum L, Niclasen B, Vollmer-Larsen A, Rasmussen HS, Graff-Larsen O. Calcitonin gene-related peptide during exercise and training. *Horm Metab Res*. 1995;27:473-475.
12. Lind H, Brudin L, Lindholm L, Edvinsson L. Different levels of sensory neuropeptides (calcitonin gene-related peptide and substance P) during and after exercise in man. *Clin Physiol*. 1996;16:73-82.
13. Jonhagen S, Ackermann P, Saartok T, Renstrom PA. Calcitonin gene related peptide and neuropeptide Y in skeletal muscle after eccentric exercise: a microdialysis study. *Br J Sports Med*. 2006;40:264-267; discussion 264-267.
14. Lechleitner P, Haring C, Mair J, Genser N, Wiedermann CJ, Dienstl F, Saria A. Exercise-induced increase in plasma concentrations of calcitonin gene-related peptide in patients with coronary heart disease and healthy controls. *International Journal of Angiology*. 1994;3:16-19.
15. Smith SA, Leal AK, Williams MA, Murphy MN, Mitchell JH, Garry MG. The TRPv1 receptor is a mediator of the exercise pressor reflex in rats. *J Physiol*. 2010;588:1179-1189.
16. Nakanishi M, Hata K, Nagayama T, Sakurai T, Nishisho T, Wakabayashi H, Hiraga T, Ebisu S, Yoneda T. Acid activation of Trpv1 leads to an up-regulation of calcitonin gene-related peptide expression in dorsal root ganglion neurons via the CaMK-CREB cascade: a potential mechanism of inflammatory pain. *Mol Biol Cell*. 2010;21:2568-2577.
17. Meng J, Ovsepian SV, Wang J, Pickering M, Sasse A, Aoki KR, Lawrence GW, Dolly JO. Activation of TRPV1 mediates calcitonin gene-related peptide release, which excites trigeminal sensory neurons and is attenuated by a retargeted botulinum toxin with anti-nociceptive potential. *J Neurosci*. 2009;29:4981-4992.
18. Dhaka A, Uzzell V, Dubin AE, Mathur J, Petrus M, Bandell M, Patapoutian A. TRPV1 is activated by both acidic and basic pH. *J Neurosci*. 2009;29:153-158.
19. Sueur S, Pesant M, Rochette L, Connat JL. Antiapoptotic effect of calcitonin gene-related peptide on oxidative stress-induced injury in H9c2 cardiomyocytes via the RAMP1/CRLR complex. *J Mol Cell Cardiol*. 2005;39:955-963.

20. Al-Rubaiee M, Gangula PR, Millis RM, Walker RK, Umoh NA, Cousins VM, Jeffress MA, Haddad GE. Inotropic and lusitropic effects of calcitonin gene-related peptide in the heart. *Am J Physiol Heart Circ Physiol*. 2013;304:H1525-1537.
21. Bell D, Schluter KD, Zhou XJ, McDermott BJ, Piper HM. Hypertrophic effects of calcitonin gene-related peptide (CGRP) and amylin on adult mammalian ventricular cardiomyocytes. *J Mol Cell Cardiol*. 1995;27:2433-2443.
22. Garciarena CD, Pinilla OA, Nolly MB, Laguens RP, Escudero EM, Cingolani HE, Ennis IL. Endurance training in the spontaneously hypertensive rat: conversion of pathological into physiological cardiac hypertrophy. *Hypertension*. 2009;53:708-714.
23. O'Connor CM, Whellan DJ, Lee KL et al. Efficacy and safety of exercise training in patients with chronic heart failure: HF-ACTION randomized controlled trial. *JAMA*. 2009;301:1439-1450.
24. Uren NG, Seydoux C, Davies GJ. Effect of intravenous calcitonin gene related peptide on ischaemia threshold and coronary stenosis severity in humans. *Cardiovasc Res*. 1993;27:1477-1481.
25. Gennari C, Nami R, Agnusdei D, Fischer JA. Improved cardiac performance with human calcitonin gene related peptide in patients with congestive heart failure. *Cardiovasc Res*. 1990;24:239-241.
26. Shekhar YC, Anand IS, Sarma R, Ferrari R, Wahi PL, Poole-Wilson PA. Effects of prolonged infusion of human alpha calcitonin gene-related peptide on hemodynamics, renal blood flow and hormone levels in congestive heart failure. *Am J Cardiol*. 1991;67:732-736.
27. Aubdool AA, Thakore P, Argunhan F et al. A Novel alpha-Calcitonin Gene-Related Peptide Analogue Protects Against End-Organ Damage in Experimental Hypertension, Cardiac Hypertrophy, and Heart Failure. *Circulation*. 2017;136:367-383.
28. Wiesel P, Mazzolai L, Nussberger J, Pedrazzini T. Two-kidney, one clip and one-kidney, one clip hypertension in mice. *Hypertension*. 1997;29:1025-1030.
29. Wang Z, Martorell BC, Walchli T, Vogel O, Fischer J, Born W, Vogel J. Calcitonin gene-related peptide (CGRP) receptors are important to maintain cerebrovascular reactivity in chronic hypertension. *PLoS One*. 2015;10:e0123697.
30. Konhilas JP, Watson PA, Maass A, Boucek DM, Horn T, Stauffer BL, Luckey SW, Rosenberg P, Leinwand LA. Exercise can prevent and reverse the severity of hypertrophic cardiomyopathy. *Circ Res*. 2006;98:540-548.
31. MaassenVanDenBrink A, Meijer J, Villalon CM, Ferrari MD. Wiping Out CGRP: Potential Cardiovascular Risks. *Trends Pharmacol Sci*. 2016;37:779-788.
32. Deen M, Correnti E, Kamm K, Kelderman T, Papetti L, Rubio-Beltran E, Vigneri S, Edvinsson L, Maassen Van Den Brink A. Blocking CGRP in migraine patients - a review of pros and cons. *J Headache Pain*. 2017;18:96.
33. Lu JT, Son YJ, Lee J, Jetton TL, Shiota M, Moscoso L, Niswender KD, Loewy AD, Magnuson MA, Sanes JR, Emeson RB. Mice lacking alpha-calcitonin gene-related peptide exhibit normal cardiovascular regulation and neuromuscular development. *Mol Cell Neurosci*. 1999;14:99-120.
34. D'Souza KM, Biwer LA, Madhavpeddi L, Ramaiah P, Shahid W, Hale TM. Persistent change in cardiac fibroblast physiology after transient ACE inhibition. *Am J Physiol Heart Circ Physiol*. 2015;309:H1346-1353.
35. Skaria T, Bachli E, Schoedon G. RSPO3 impairs barrier function of human vascular endothelial monolayers and synergizes with pro-inflammatory IL-1. *Mol Med*. 2018;24:45.
36. Dorn GW, 2nd, Robbins J, Sugden PH. Phenotyping hypertrophy: eschew obfuscation. *Circ Res*. 2003;92:1171-1175.
37. Vega RB, Konhilas JP, Kelly DP, Leinwand LA. Molecular Mechanisms Underlying Cardiac Adaptation to Exercise. *Cell Metab*. 2017;25:1012-1026.
38. Piek A, de Boer RA, Sillje HH. The fibrosis-cell death axis in heart failure. *Heart Fail Rev*. 2016;21:199-211.

39. Yokoyama U, Patel HH, Lai NC, Aroonsakool N, Roth DM, Insel PA. The cyclic AMP effector Epac integrates pro- and anti-fibrotic signals. *Proceedings of the National Academy of Sciences*. 2008;105:6386-6391.
40. Phosri S, Arieyawong A, Bunrukchai K, Parichatikanond W, Nishimura A, Nishida M, Mangmool S. Stimulation of Adenosine A2B Receptor Inhibits Endothelin-1-Induced Cardiac Fibroblast Proliferation and α-Smooth Muscle Actin Synthesis Through the cAMP/Epac/PI3K/Akt-Signaling Pathway. *Frontiers in Pharmacology*. 2017;8
41. Russell FA, King R, Smillie SJ, Kodji X, Brain SD. Calcitonin gene-related peptide: physiology and pathophysiology. *Physiol Rev*. 2014;94:1099-1142.
42. Courilleau D, Bisserier M, Jullian JC, Lucas A, Bouyssou P, Fischmeister R, Blondeau JP, Lezoualc'h F. Identification of a tetrahydroquinoline analog as a pharmacological inhibitor of the cAMP-binding protein Epac. *J Biol Chem*. 2012;287:44192-44202.
43. Aveseh M, Koushkie-Jahromi M, Nemati J, Esmaeili-Mahani S. Serum calcitonin gene-related peptide facilitates adipose tissue lipolysis during exercise via PIPLC/IP3 pathways. *Endocrine*. 2018;61:462-472.
44. Chapter 3. Principles of treatment. *Hypertension Research*. 2009;32:24.
45. Hegde SM, Solomon SD. Influence of Physical Activity on Hypertension and Cardiac Structure and Function. *Curr Hypertens Rep*. 2015;17:77.
46. Lear SA, Hu W, Rangarajan S et al. The effect of physical activity on mortality and cardiovascular disease in 130 000 people from 17 high-income, middle-income, and low-income countries: the PURE study. *Lancet*. 2017;390:2643-2654.
47. Hasbak P, Saetrum Opgaard O, Eskesen K, Schifter S, Arendrup H, Longmore J, Edvinsson L. Investigation of CGRP receptors and peptide pharmacology in human coronary arteries. Characterization with a nonpeptide antagonist. *J Pharmacol Exp Ther*. 2003;304:326-333.
48. Li J, Levick SP, DiPette DJ, Janicki JS, Supowit SC. Alpha-calcitonin gene-related peptide is protective against pressure overload-induced heart failure. *Regul Pept*. 2013;185:20-28.
49. Supowit SC, Rao A, Bowers MC, Zhao H, Fink G, Steficek B, Patel P, Katki KA, Dipette DJ. Calcitonin gene-related peptide protects against hypertension-induced heart and kidney damage. *Hypertension*. 2005;45:109-114.
50. Yallampalli C, Dong YL, Wimalawansa SJ. Calcitonin gene-related peptide reverses the hypertension and significantly decreases the fetal mortality in pre-eclampsia rats induced by N(G)-nitro-L-arginine methyl ester. *Hum Reprod*. 1996;11:895-899.
51. Fujioka S, Sasakawa O, Kishimoto H, Tsumura K, Morii H. The antihypertensive effect of calcitonin gene-related peptide in rats with norepinephrine- and angiotensin II-induced hypertension. *J Hypertens*. 1991;9:175-179.
52. Chung E, Leinwand LA. Pregnancy as a cardiac stress model. *Cardiovasc Res*. 2014;101:561-570.
53. Gangula PR, Wimalawansa SJ, Yallampalli C. Pregnancy and sex steroid hormones enhance circulating calcitonin gene-related peptide concentrations in rats. *Hum Reprod*. 2000;15:949-953.
54. Peixoto-Neves D, Soni H, Adebiyi A. CGRPergic Nerve TRPA1 Channels Contribute to Epigallocatechin Gallate-Induced Neurogenic Vasodilation. *ACS Chem Neurosci*. 2018
55. Wang Y, Wang DH. TRPV1 Ablation Aggravates Inflammatory Responses and Organ Damage during Endotoxic Shock. *Clinical and Vaccine Immunology*. 2013;20:1008-1015.
56. Gao F, Lv TR, Zhou JC, Qin XD. Effects of obesity on the healing of bone fracture in mice. *J Orthop Surg Res*. 2018;13:145.
57. Leggio M, Mazza A, Cruciani G, Sgorbini L, Pugliese M, Bendini MG, Severi P, Jesi AP. Effects of exercise training on systo-diastolic ventricular dysfunction in patients with hypertension: an echocardiographic study with tissue velocity and strain imaging evaluation. *Hypertens Res*. 2014;37:649-654.
58. Tepper SJ. History and Review of anti-Calcitonin Gene-Related Peptide (CGRP) Therapies: From Translational Research to Treatment. *Headache*. 2018;58 Suppl 3:238-275.

FIGURE LEGENDS

Figure 1. Survival and causes of death after 1K1C surgery. (A) Kaplan-Meier survival curves of WT and αCGRP-/- mice following 1K1C. Comparison was performed with the Mantel-Cox test. (B) Incidences of fatal events diagnosed by autopsy histopathology in WT and αCGRP-/- mice that were euthanized due to signs of kidney failure (KF) or died suddenly without signs of impaired well-being following 1K1C. Dilated cardiac phenotype (DCP) was observed only in αCGRP-/- mice. 1K1C: one-kidney-one-clip, AR: aortic rupture, PRD: presumed cardiac rhythm disturbance.

Figure 2. Basic cardiac variables in chronically hypertensive WT and αCGRP-/- mice. (A) Relative wall thickness (RWT), and (B) heart weight to tibia length ratio (HW/TL). Dashed lines indicate the upper 95% confidence interval of the mean of RWT (A) or HW/TL (B) of sham-operated WT (green, n=9) and αCGRP-/- (black, n=9) mice. Dot color indicates hypertensive mice with increased (blue) or decreased (red) RWT compared with respective sham-operated mice. (C) Proportion of animals with echocardiographically based classification of concentric hypertrophy (CH) and dilated cardiac phenotype (DCP) in each study group. (D) Systolic and diastolic blood pressure did not differ significantly between different hypertension experimental groups though tended to be lower in mice exhibiting DCP. Means ±S.E.M., One-way ANOVA, Bonferroni post-hoc test of concentric hypertrophy only and applied to (D) only. sed: sedentary.

Figure 3. Exercise performance and exercise-induced plasma αCGRP concentration in chronically hypertensive WT and αCGRP-/- mice. (A) Total distance, (B) average daily distance and (C) average speed run by chronically hypertensive WT and αCGRP-/- mice over 4 weeks study period. αCGRP treatment significantly increased voluntary wheel running activity in WT mice. Antagonist (CGRP8-37) treatment had no effect compared with vehicle treatment. Vehicle-treated αCGRP-/- mice run significantly less compared with vehicle-treated WT mice. αCGRP treatment increased running distance also in αCGRP-/- mice. Means ±S.E.M., Kruskal-Wallis, Dunn's post-hoc test of concentric hypertrophy only. Refer Online Table IV for statistics involving whole group means. Plasma αCGRP concentration immediately after 7 minutes of voluntary wheel running in naive normotensive (D, n=6) and vehicle-treated hypertensive (E, n=7) WT mice. Means ±S.E.M., unpaired t-test. sed: sedentary, CH: concentric hypertrophy, DCP: dilated cardiac phenotype.

Figure 4. Cardiomyocyte hypertrophy in chronically hypertensive WT and αCGRP-/- mice. (A) αCGRP deficiency or αCGRP receptor antagonism increased cardiomyocyte cross-sectional area (CSA) while αCGRP treatment reversed CSA. Dashed lines indicate the mean cardiomyocyte CSA of sham-operated WT (green, n=9) and αCGRP-/- (black, n=9) mice. Data are means ±S.E.M of 75 cells in each group, One-way ANOVA, Bonferroni post-hoc test of concentric hypertrophy only. Refer Online Table V for statistics involving whole group means. (B) Representative CF^{TM}488A-conjugated wheat germ agglutinin-stained (green) myocardial sections of (A). Cell nuclei in blue. sed: sedentary, CH: concentric hypertrophy, DCP: dilated cardiac phenotype.

Figure 5. Ejection fraction (EF), and fractional shortening (FS) in chronically hypertensive WT and αCGRP-/- mice. Dashed lines indicate the mean of EF (A) or FS (B) of sham-operated WT (green, n=9) and αCGRP-/- (black, n=9) mice. In WT mice, voluntary running improved cardiac function which was abolished by treatment with antagonist (CGRP8-37). Whereas voluntary running alone did not significantly increase cardiac function in hypertensive αCGRP-/- mice, αCGRP treatment did. Additional voluntary running did not further improve EF and FS (αCGRP-/-, run, αCGRP vs αCGRP-/-, sed, αCGRP). Complete echocardiography data are shown in Online Table VI. One-way ANOVA, Bonferroni post-hoc test of concentric hypertrophy only. Refer Online Table VII for statistics involving whole group means. sed: sedentary, CH: concentric hypertrophy, DCP: dilated cardiac phenotype.

Figure 6. Fetal gene expression signature in chronically hypertensive WT and αCGRP-/- mice. mRNA expression levels (log_2-transformed) of (A) myosin heavy chain-6 (Myh6), (B) myosin heavy chain-7 (Myh7), (C) atrial natriuretic peptide (Nppa), (D) brain natriuretic peptide (Nppb), and (E) sarcoplasmic reticulum Ca^{2+} ATPase-2 (SERCA2) in cardiac tissues of sedentary and voluntarily running chronically hypertensive mice. Dashed lines indicate the mean expression level of respective mRNA in sham-operated WT (green, n=9) and αCGRP-/- (black, n=9) mice. Global αCGRP deficiency or αCGRP receptor antagonism reduced Myh6 and SERCA2 expression while simultaneously increasing expression of Myh7, Nppa and Nppb. Data are means ±SD, One-way ANOVA, Bonferroni post-hoc test of concentric hypertrophy only. Refer Online Table VIII for statistics involving whole group means. sed: sedentary, CH: concentric hypertrophy, DCP: dilated cardiac phenotype.

Figure 7. Myocardial collagen expression and fibrosis in chronically hypertensive WT and αCGRP-/- mice. mRNA expression levels (log_2-transformed) of (A) collagen type-1 α1 (Col1a1) and (B) collagen type-3 α1 (Col3a1), and (C) total collagen protein were reduced by voluntary running or αCGRP treatment in hypertensive WT mice, an effect that was inhibited by antagonist (CGRP8-37) treatment. Exercise did not reverse the pro-fibrotic phenotype in vehicle-treated αCGRP-/- mice. In contrast, αCGRP treatment considerably reduced Col1a1 and Col3a1 expression, and total collagen protein in αCGRP-/- hearts. Dashed lines indicate the mean expression level of respective targets in sham-operated WT (green, n=9) and αCGRP-/- (black, n=9) mice. Data are means ±S.E.M, One-way ANOVA, Bonferroni post-hoc test of concentric hypertrophy only. Refer Online Table VIII for statistics involving whole group means. These observations were confirmed by van Gieson's staining as shown in panel (D). sed: sedentary. CH: concentric hypertrophy, DCP: dilated cardiac phenotype.

Figure 8. Effect of αCGRP treatment on myofibroblast differentiation and proliferation of cardiac fibroblasts (CF). (A) cAMP levels in murine CF treated with either αCGRP alone or αCGRP+CGRP8-37 for 20 min. (B) mRNA expression levels of collagen type-1 α1 (Col1a1) and collagen type-3 α1 (Col3a1), and (C) collagen protein in CF treated in the presence or absence of cAMP synthesis inhibitor 2'5-DDA and EPAC1 inhibitor CE3F4 as indicated for 48 h (B) or 72 h (C). (D) Immunofluorescence staining of collagen in CF treated as indicated for 72 h. Blue, cell nuclei. Negative control was obtained by omitting the primary antibody. Microphotographs represent triplicates of three independent experiments. (E) Proliferative capacity of CF treated in the presence or absence of 2'5-DDA and CE3F4 as indicated for 36 h. (F) EPAC1 mRNA and protein concentrations in CF treated as indicated for 12 h (mRNA) or 24 h (protein). Taken together, αCGRP specifically inhibits myofibroblast differentiation and proliferation of CF through cAMP effector EPAC1. Means ±S.E.M of three (A, B, C, F) or five (E) independent experiments run in triplicates, One-way ANOVA, Bonferroni post-hoc test.

Novelty and Significance

What Is Known?

- Exercise has cardioprotective effects in chronic hypertension.
- Plasma alpha calcitonin gene-related peptide (αCGRP) concentration is increased during exercise due to spill over from neurons innervating working skeletal muscles and promotes physiological cardiomyocyte growth.
- When given in vasodilatory or blood pressure normalizing dosages, αCGRP and αCGRP-analogues inhibit hypertension-induced heart damage.

What New Information Does This Article Contribute?

- Basal, plasma concentrations of endogenous αCGRP are essential for maintaining cardiac function in chronic sustained hypertension.
- Cardioprotective effects of exercise in chronic persistent hypertension are mediated through endogenous αCGRP signaling.

Regular physical activity or exercise alleviates pathological cardiac remodeling and is therefore recommended as a key component of cardiac rehabilitation in chronic hypertensive patients. The neuropeptide αCGRP has causative roles in migraine and is released by exercising skeletal muscles. It has positive chronotropic and inotropic effects and stimulates physiological cardiomyocyte growth. Infusion of αCGRP can acutely improve cardiac function in congestive heart failure and hypertension. We demonstrate here for the first time that basal, exercise-independent endogenous αCGRP is crucial (i) for suppressing pathological cardiac growth, myocardial fetal gene reactivation and interstitial fibrosis as well as (ii) for preserving cardiac function in chronic hypertension, independent of lowering the pathologically elevated blood pressure. Therefore, inhibition of endogenous αCGRP, currently approved as prophylactic migraine therapy, may adversely affect cardiac function in individuals who already have hypertension, or may develop hypertension later in life. Further, we found that the cardioprotective effects of exercise in hypertension are αCGRP-dependent. Hence, αCGRP antagonists may invalidate exercise recommendations in chronic hypertensive patients. On the other hand, αCGRP agonism may be an alternative therapeutic strategy to mimic cardioprotective effects of exercise in clinical conditions where patients are mobility impaired or exercise is otherwise contraindicated.

Figure 1

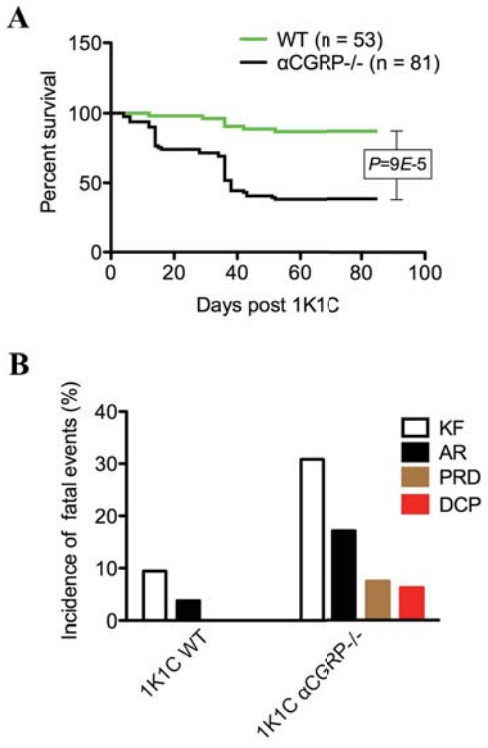


Figure 2

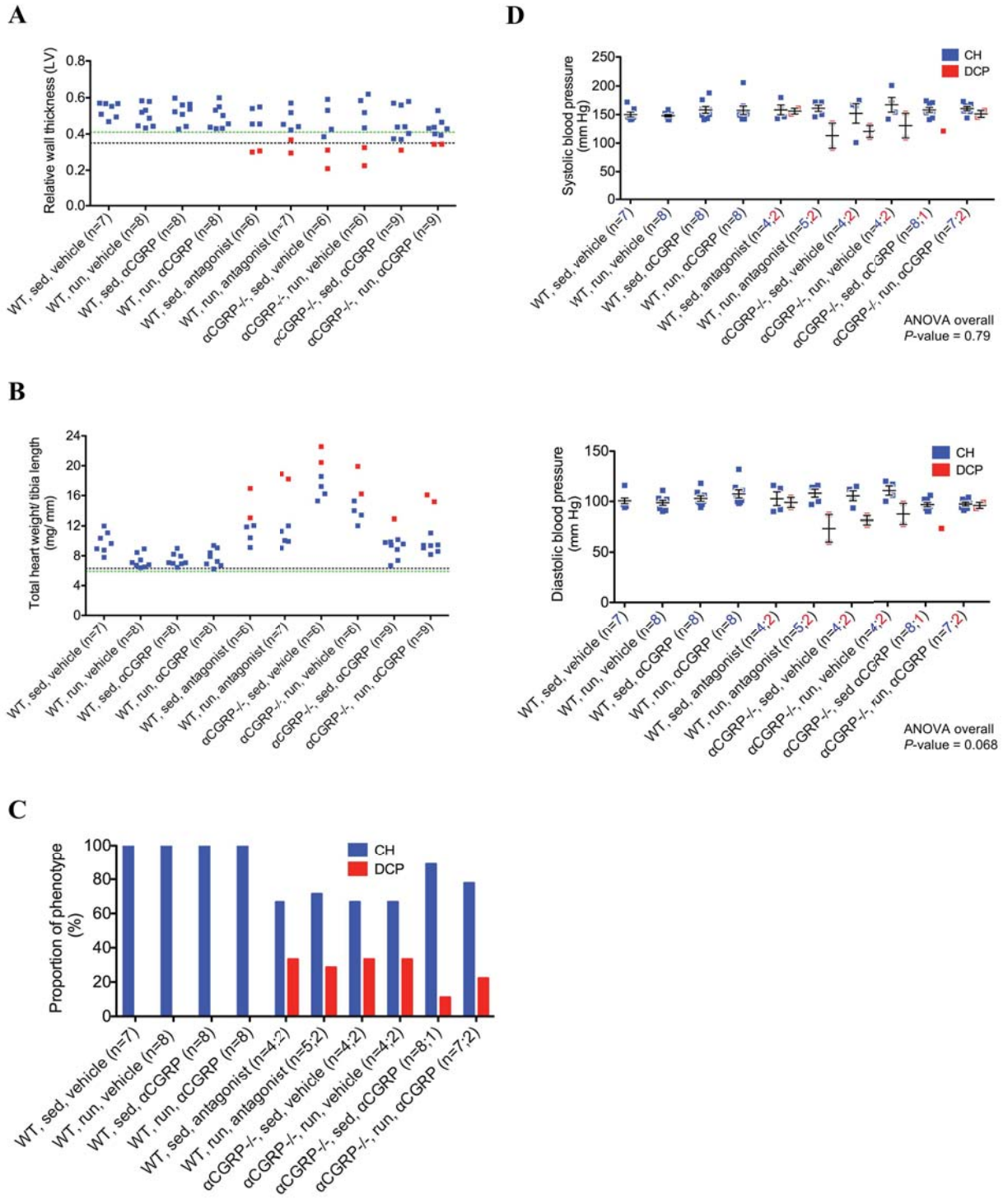


Figure 3

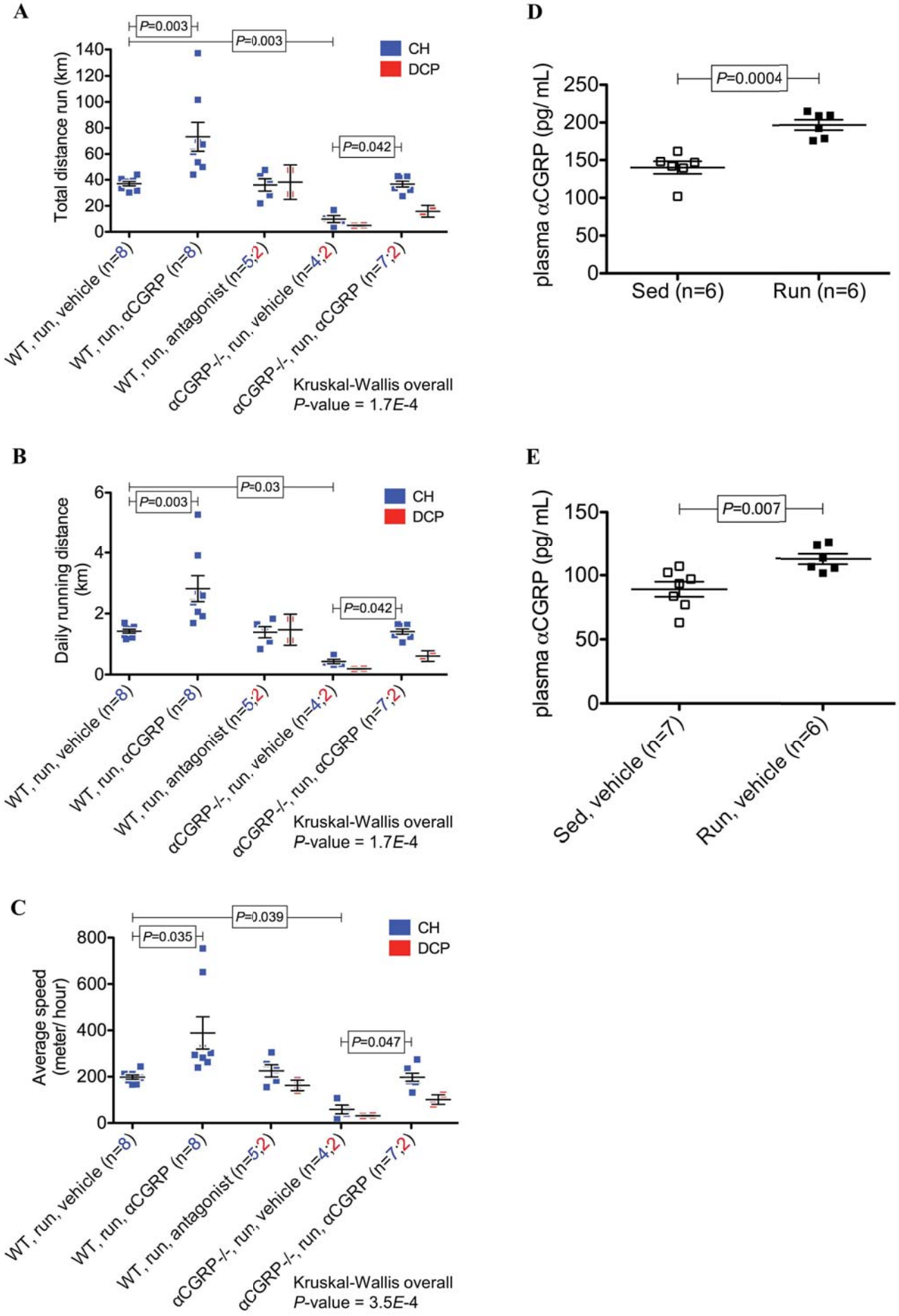


Figure 4

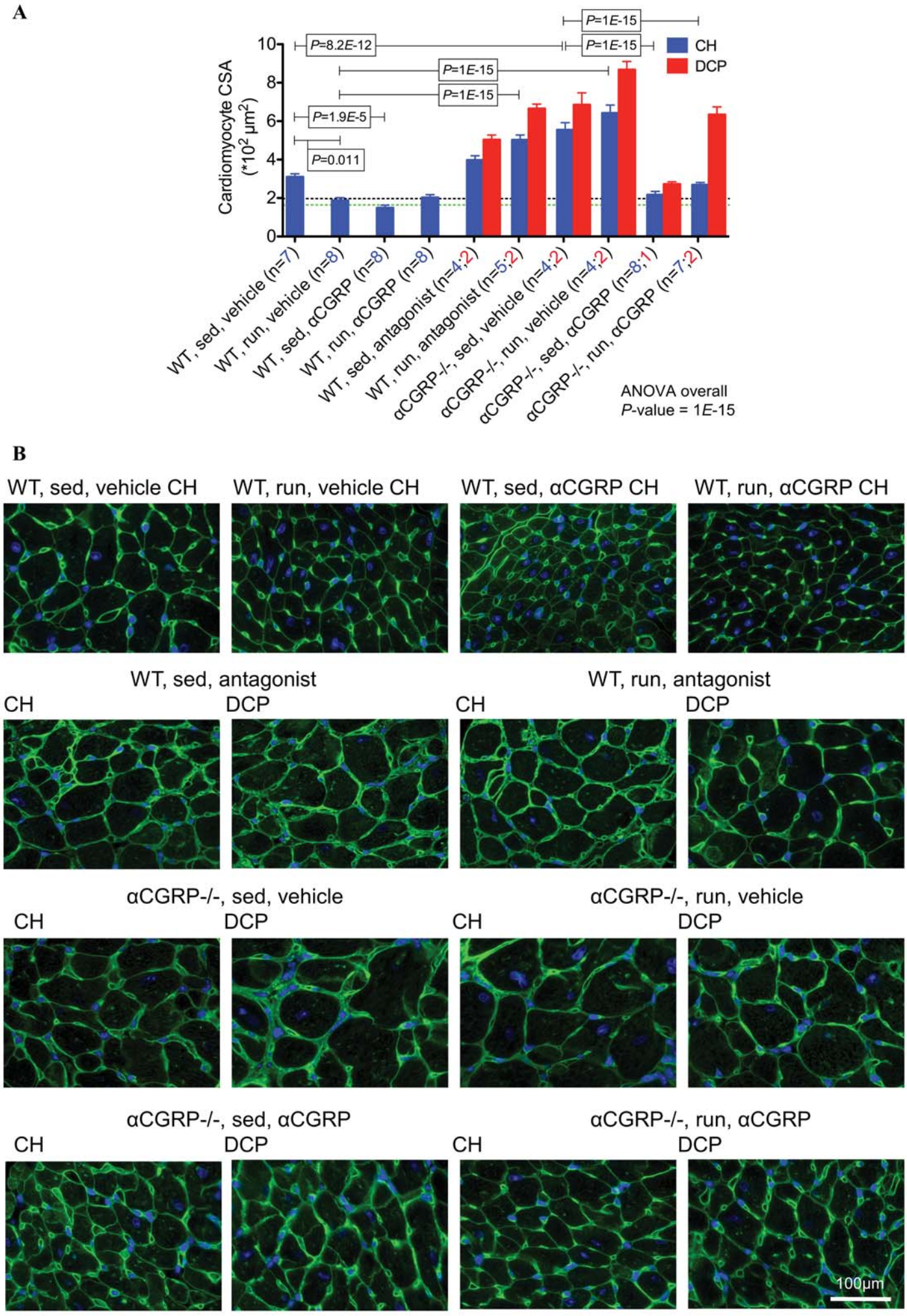


Figure 5

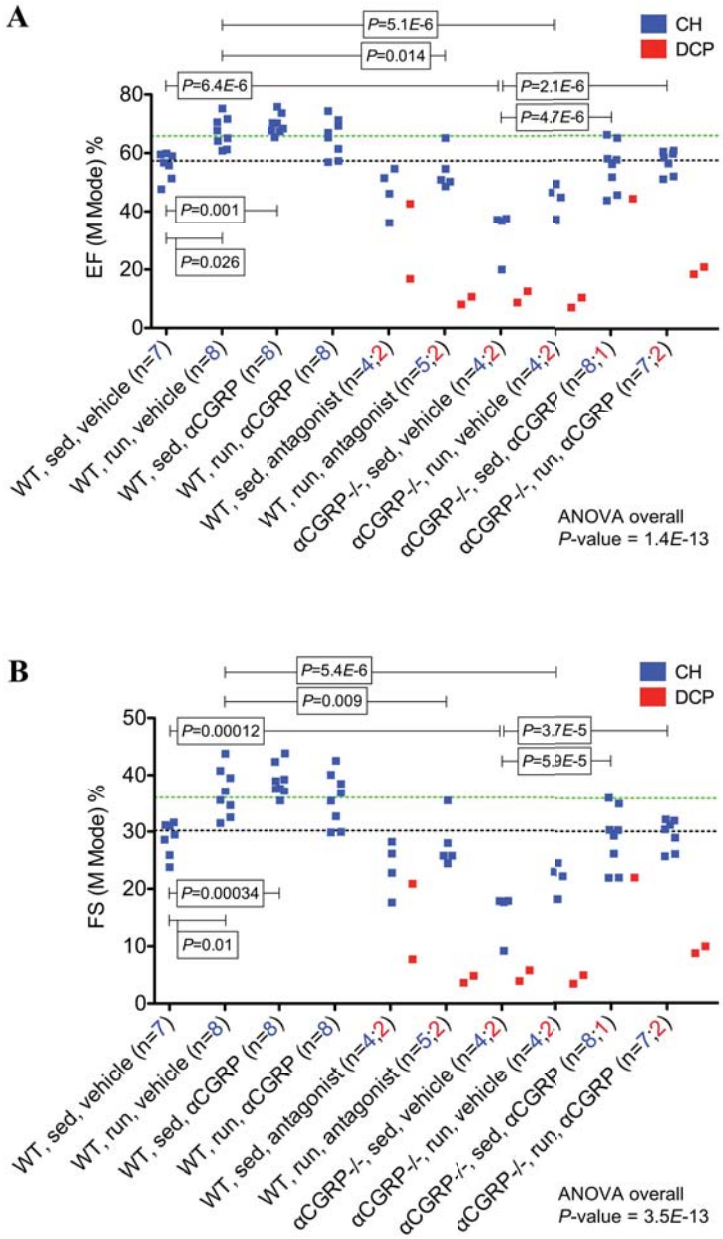
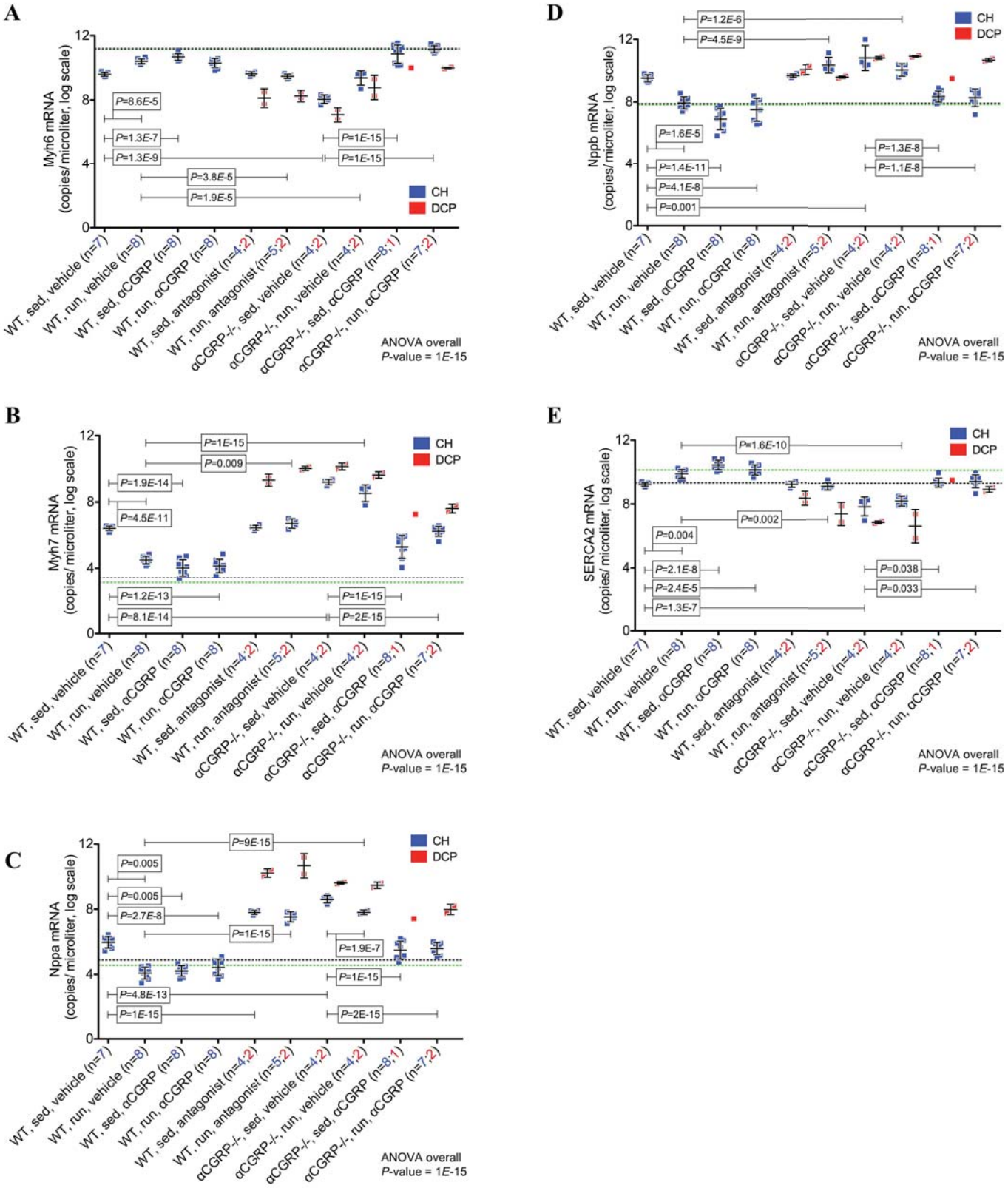


Figure 6



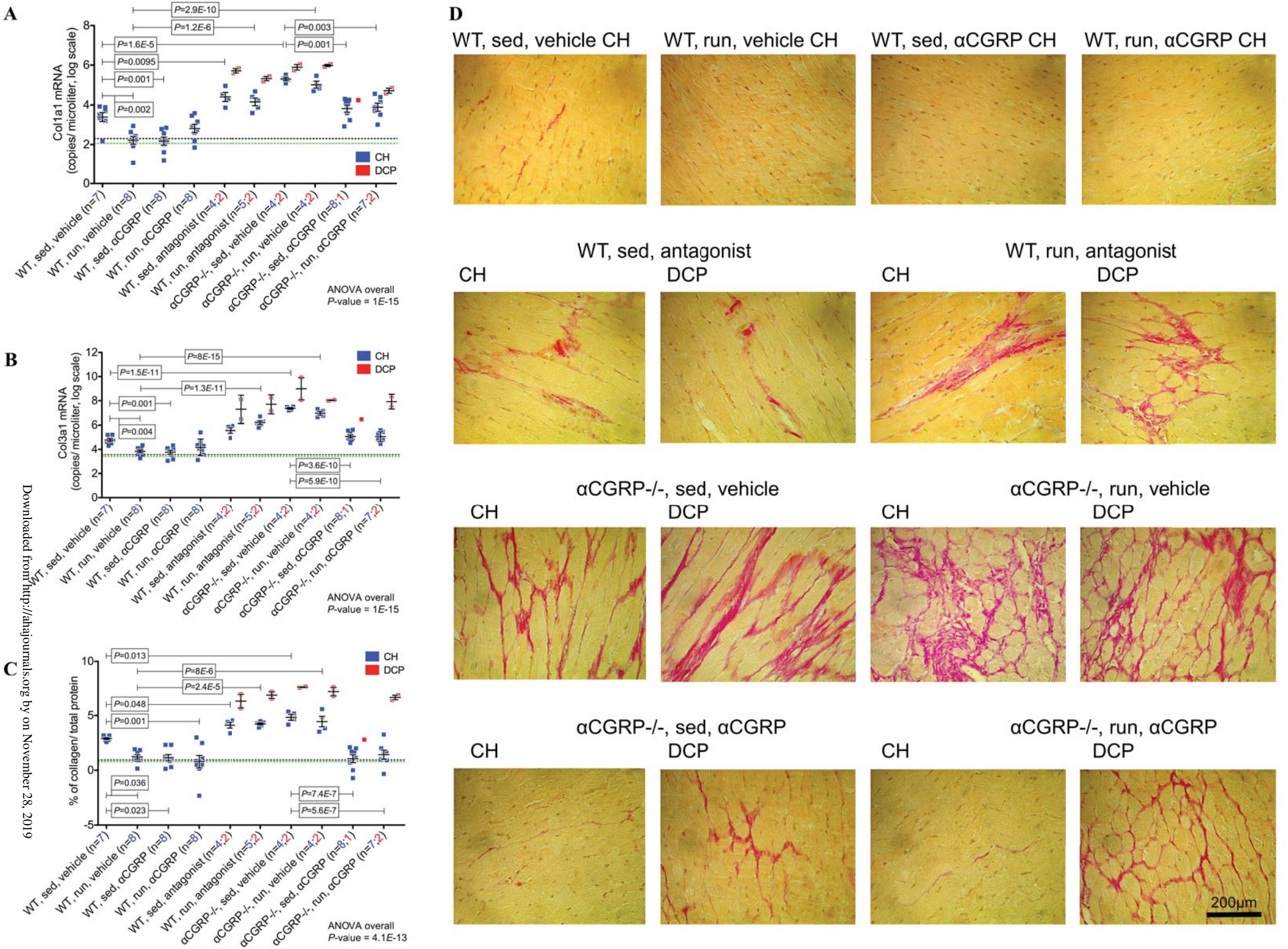


Figure 8